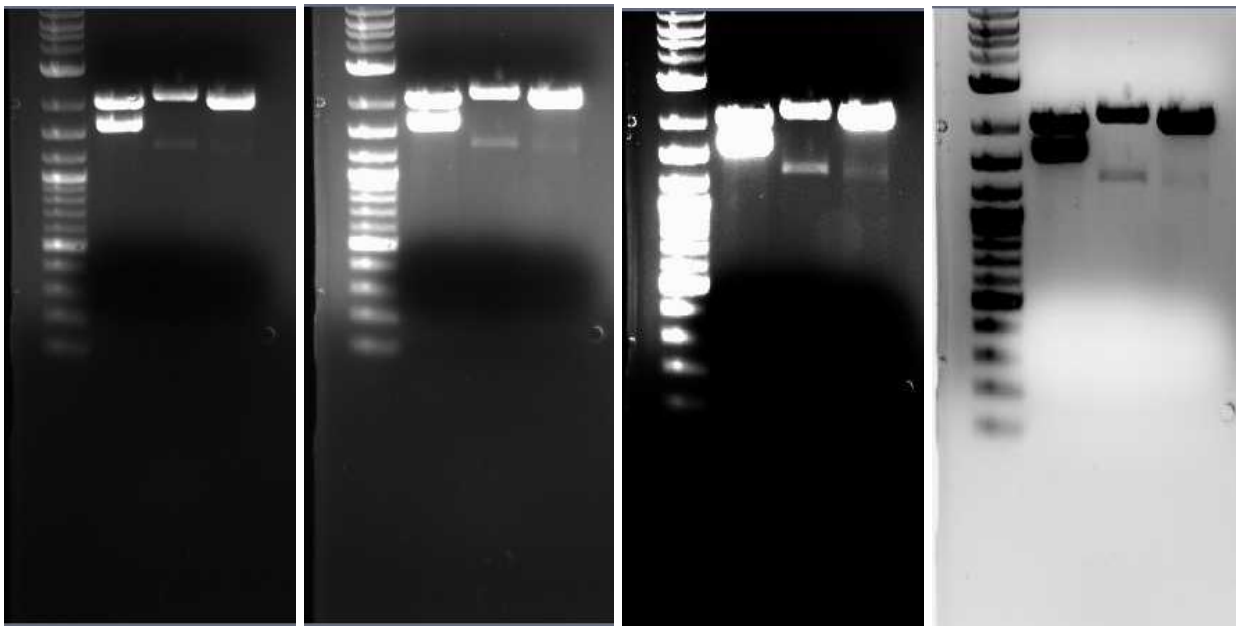
Day 25 - Monday - 07/07/14

- Kayla and Mike worked on digesting pieces of the banana odor construct because the sequencing results for part 22A showed that the plasmid had ligated to itself and did not contain the desired construct. 25 uL digests for parts 8C, 16K, and 21C were set up. The digest tubes contained 10 uL of miniprep DNA, 9.5 uL of dH2O, 2.5 uL of NEB Buffer 2, 1 uL of BSA, 1 uL of EcoRI, and 1 uL of PstI. The digest tubes were placed in the 37°C water bath for two hours. Following the two hour digest period, 5 uL of 6x loading buffer were added to each tube. 10 uL of standard ladder and 15 uL of each sample were loaded in a gel. The gel was run at 72V for one hour. Four pictures of the gel can be seen below. Each picture has a different UV exposure time. The insert sizes for 8C, 16K, and 21 C should be 1581, 1710, and 1722 bp respectively. The insert sizes for 16K and 21C seem to be the right size, but there is not a smaller band present in the 8C digest lane.

7/7/14 Gel Photos



Gel Key

Lane 1	Ladder
Lane 2	8C Digested with E+P
Lane 3	16K Digested with E+P
Lane 4	21C Digested with E+P

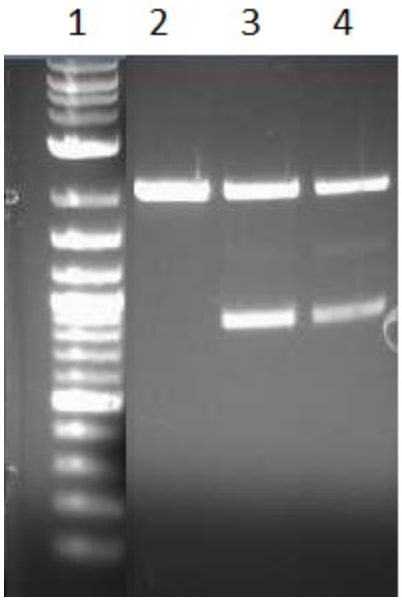
In addition to the gel, Mike prepared a new liquid culture of part 17K from the glycerol stock. Kayla resuspended part J45199 from well 5I on iGEM well plate 4 and performed a transformation.

- Corbyn and Shawna started the day by annealing the oligos of RBS, so we would be able to use them in another attempt to create the RBS-Promoter construct. Per professor Farny's request, we also created a 50uL E-P digest of the company-provided CAEV and left it to digest at 37degrees overnight. We also digested the annealed RBS (X-P) [this was a 50uL digest as well], promoter (E-S), and the Chloramphenicol backbone (E-P). Then, we ligated the RBS and promoter into the chloramphenicol backbone. We used the following ratios for the ligations:

Tube Label	Amount of RBS (uL)	Amount of Promoter (uL)
pr	2	2
prA	1	2
prB	2	1

The three ligations were then transformed and plated and left in the warm room overnight. Also, we sent BCLA clone 3 and 5 for sequencing.

- Alex and Chloe prepared liquid cultures from 5 colonies from each plate of the interlab ligations. New test digestions were also prepared for the interlab ligations already made to double check if inserts were present and were run on the following gel:



Lane	Contents
1	Ladder
2	18C+19C (1)
3	18C+19Cn (2)
4	19C+20C

Then, 5 different colonies were picked from each interlab ligation to be cultured in liquid media.

Day 26 - Tuesday - 07/08/14

The team had their weekly meeting, and we also had a visitor from a local high school iGEM team that had tried to enter a project but was unsuccessful in doing so. We spoke to her about different ways to raise money as well as showed her our lab space. We are considering a collaboration (big sister/brother) kind of collaboration with the local high school team.

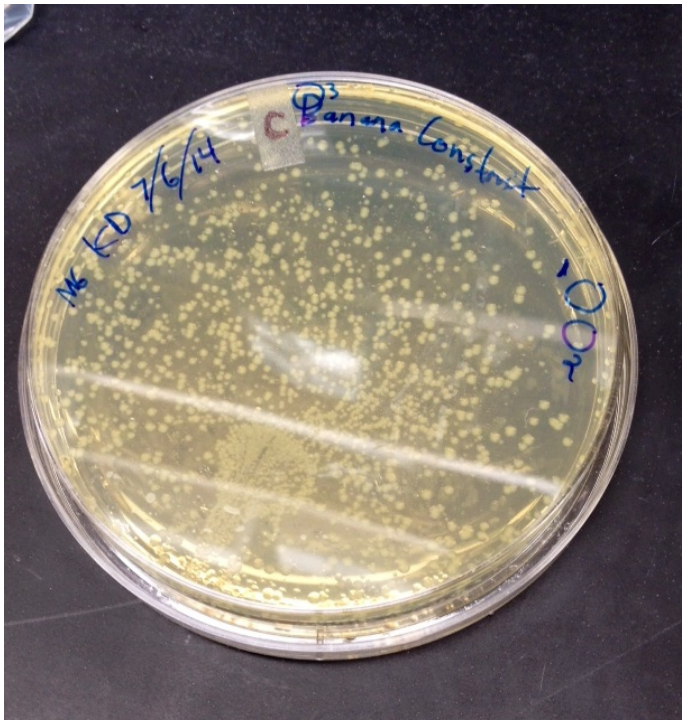
- Alex and Chloe confirmed that the previous interlab ligations had failed using the plate reader and 5 colonies from each ligation plate that were cultured in liquid media. 50 ul digests for both constructs for the interlab study were also performed. The digest volumes are shown in the following table:

	18C (Promoter + Backbone)	**20C (Low Promoter + Backbone)**	**19C (RBS-GFP-DT)**
Water	20 ul	20 ul	20 ul
DNA	20 ul	20 ul	20 ul
BSA	1 ul	1 ul	1 ul
NEB 2	5 ul	5 ul	5 ul
S	2 ul	2 ul	
P	2 ul	2 ul	
X		2 ul	2 ul

  The digests of 18C and 20C, intended to become vectors with the promoter still attached, were also treated with 1 uL of CIP.
- Kayla and Mike started off the day by checking the transformation plate for the iGEM RBS/ATF1/DT construct. Many colonies grew on the chloramphenicol plate, as seen in the photo below. After the plate was a checked, a miniprep was made from the 17K overnight liquid culture. According to the NanoDrop, the DNA concentration of the miniprep was only 46.9 ng/uL. Because this was such a low DNA concentration, a new liquid culture was prepared from the 17K glycerol stock. Three liquid cultures were also prepared for the new RBS/ATF1/DT construct using three colonies from the transformation plate. Finally, the miniprep for part 8C was sent off for sequencing to see if it did in fact contain the ATF1 insert. Based on the gel from yesterday, the 8C miniprep did appear to have the ATF1 insert, while minipreps 16K and 21C did not have an insert of the correct length. The vector may have a cryptic similar to that of a restriction site that

resulted in restriction enzymes cutting at the incorrect site and producing the smaller, more faint bands that appeared on the gel.

BBa_J45199 Transformation Plate

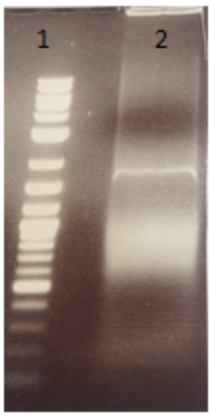


- Corbyn and Shawna looked at the sequencing of the BCLA biobrick and it was very inaccurate. It appeared as if there was no BCLA insert in the cam vector, but it had previously shown on a gel. We think this problem in sequencing was due to using the wrong primers (prefix and suffix) instead of using VF and VR. We collected the promoter and RBS plates that had been in the warm room overnight, and the number of colonies that grew on each can be seen in the table below. Also, we posted pictures of the plates in this week's plate pictures folder.

Plate	***Number of Colonies***
2 RBS 2 Promoter	>100
1 RBS 2 Promoter	~15
2 RBS 1 Promoter	~15

We also ran the entire (50uL) E-P overnight digest of CAEV on a 1% agarose gel at 60V with the hope of gel purifying the CAEV insert. In order to fit all 60uL of the sample into the wells, we created a special gel with 2 wells merged into one. A picture of the gel with labeled lanes can be

seen below. As you can see, the band was extremely fuzzy and we were unable to gel purify the CAEV.



Lane	Contents
1	10uL Ladder
2	50uL E-P CAEV digest

At the end of the day, Corbyn and Shawna liquid cultured 3 colonies of CAEV from the original plate and 5 colonies from the promoter-RBS ligation. These were then placed in the 37degree shaker overnight.

Day 27 - Wednesday- 07/09/14

- Alex and Chloe gel purified the interlab study digested parts. Then, a series of ligations was performed, including a control, in order to test which ratios of vector to plasmid would be the most effective.

Vector (18C)	1 uL	1 uL	1uL	1uL	1uL
Insert (19C)	0 uL	10 uL	1 uL	2 uL	5 uL
Water	16 uL	6 uL	15 uL	14 uL	12 uL
Buffer	2 uL	2 uL	2 uL	2 uL	2 uL
Ligase	1 uL	1 uL	1 uL	1 uL	1 uL

Vector (20C)	7 uL	7 uL	7 uL	7 uL	7 uL
Insert (19C)	0 uL	10 uL	1 uL	2 uL	5 uL
Water	10 uL	0 uL	9 uL	8 uL	6 uL
Buffer	2 uL	2 uL	2 uL	2 uL	2 uL
Ligase	1 uL	1 uL	1 uL	1 uL	1 uL

The ligations were then transformed and left to incubate overnight. An agglutination assay was also conducted and left to sit overnight.

- Kayla and Mike processed minipreps of the four liquid cultures made the previous day, the three minipreps from the colonies yielded good concentrations, but the miniprep from the 17K liquid stock was not sufficient with a jagged curve. The results are below:

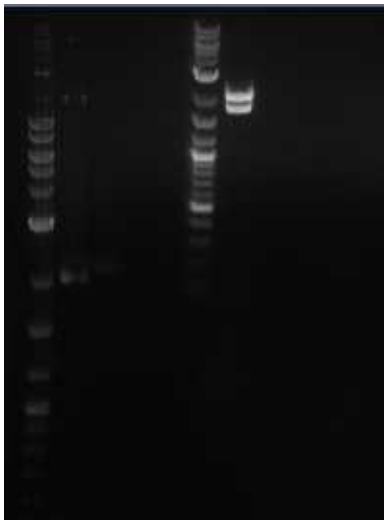
Miniprep Name	Origin	Concentration (ng/ul)
23C mp1	23C Transformation Col1	365.8
23 Cmp2	23C Transformation Col2	316.6
23C mp3	23C Transformation Col3	290.3

- As part of chosen option one (In the Week 6 directory) Kayla and Mike made four separate digests, one of the RBS-ATF1-DT from 23C mp1 with X+P, one from 7C (pARS) with E+S, one from the kan vector with E+P, and another Kan vector with E+P along with CIP. With these digests, 10 ligation reactions were prepared. Four of the ligations were prepared with the CIP+ vector, while four were prepared with the CIP- vector. The two sets of five ligations share the characteristics listed in the table below.

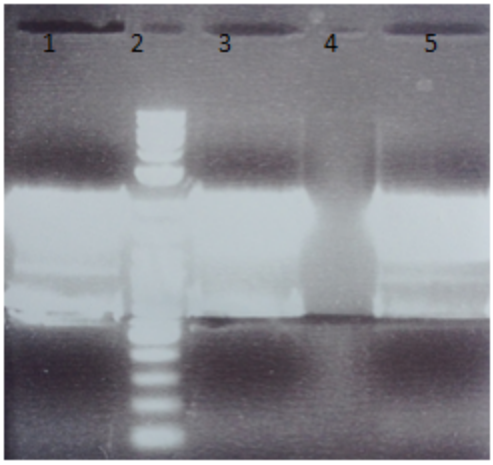
Ligation Designation	**A**	**B**	**C**	**D**	**E**
Vector (either CIP or non-CIP)[ul]	2	1	2	2	2
23C (RBS-ATF1-DT)[ul]	2	1	1	2	0
7C (pARS)[ul]	2	1	1	1	0
ligase buffer[ul]	2	2	2	2	2
dH2O[ul]	11	14	13	12	15
ligase[ul]	1	1	1	1	1

The ligation DNA was then used to perform 10 different transformations. The ligations are expected to work because the insert sizes were confirmed by the running of the digests. In the gel below, the band at about 500 bp can be seen in the second lane containing the arsenic promoter digest. In the fourth lane, a band for the RBS-ATF1-DT construct can be seen at about 1.7 kb.

7/9/14 Test Digest

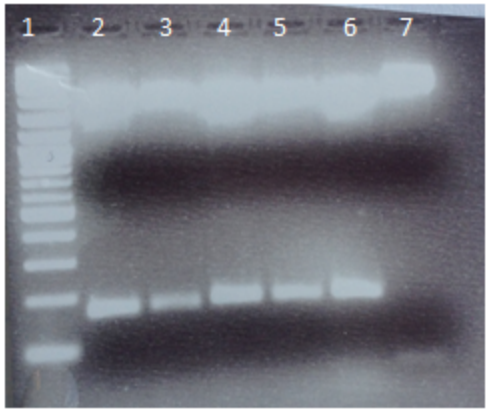


- In the morning, Corbyn and Shawna miniprepped the 3 CAEV cultures and the 5 RBS+Promoter cultures from the night before. We then checked the DNA concentration using the nanodrop, and all of the concentrations were fine. We then prepared numerous large 50uL digests for 2.5 hours in order to run a gel and check the correctness of the inserts. The 5 promoter+RBS constructs were digested with E-P, the 3 CAEV minipreps was digested with E-P as well, and finally, as a control for the Promoter+RBS gel, promoter was digested with E-S (to cut out the mRFP). The three CAEV digestions we then run at 61V on 1% gel for about an hour, once again using the larger wells and all 50uL of the digest. A picture of the resulting gel with labeled lanes can be seen below, but it is a picture showing the gel after we removed a portion of the CAEV insert bands for gel purification.



Lane	Contents
1	50uL CAEV 1
2	ladder
3	50uL CAEV 2
4	empty
5	50uL CAEV 3

After the gel purification, we nano dropped the three samples and all had DNA concentrations around .02ng/uL, which is normal for gel purification. On another gel (2.5%), we ran all 5 promoter+RBS digestions along with the E-S promoter digestions as a control. This was also at 61V and for about an hour. A picture of the resulting gel can be seen below, and it shows promise for being the correct size band for a promoter-RBS complex.



Lane	Contents
1	ladder
2	Promoter +RBS 1
3	Promoter +RBS 2
4	Promoter +RBS 3
5	Promoter +RBS 4
6	Promoter +RBS 5
7	Promoter (E-S)

At the end of the day, we also liquid cultured BCLA biobrick 3 and 5 from the glycerol stock with the intention of another miniprep and then using that to sequence.

Day 28 - Thursday - 07/10/14

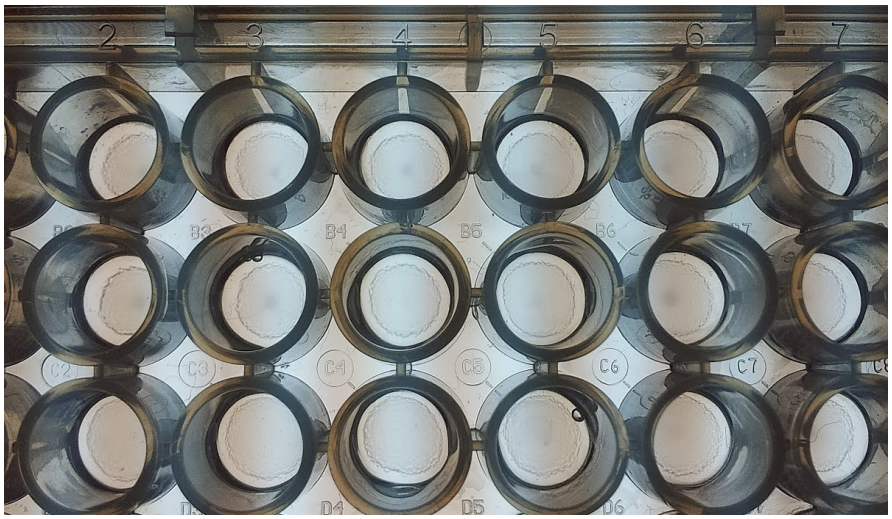
- Alex and Chloe analyzed the Agglutination assay from the previous day. The contents of the plate are shown in the following table:

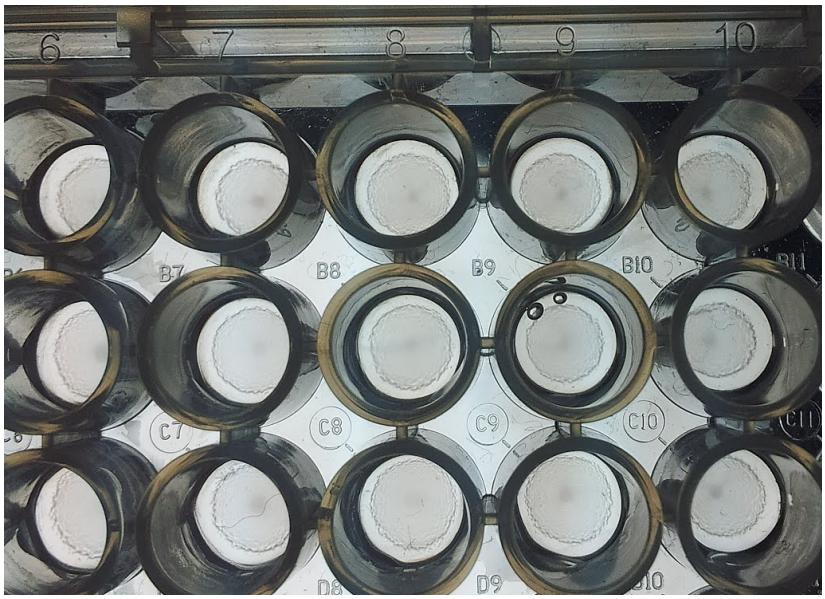
Antibody Conc.	**1:50**	**1:100**	**1:200**	**1:400**	**1:800**	**1:1600**	**1:3200**	**1:6400**	**1:12800**	**1:25600**	**1:51200**
No Ab Ctrl	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot
Nonspecific Ab Ctrl	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot

E. coli Ab	Film	Film	Film	Film	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot	Gray dot

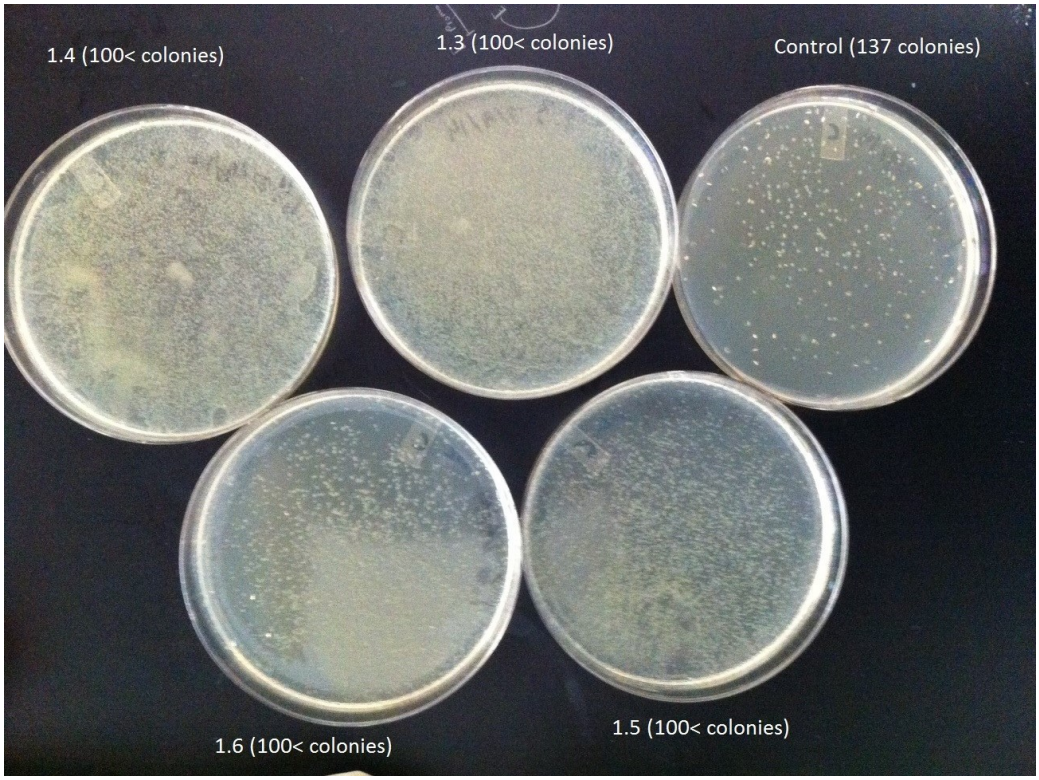
Pictures of the wells were taken. (However, the nature of the wells cannot be seen very accurately through the pictures taken with this setup. A different setup should probably be found.)

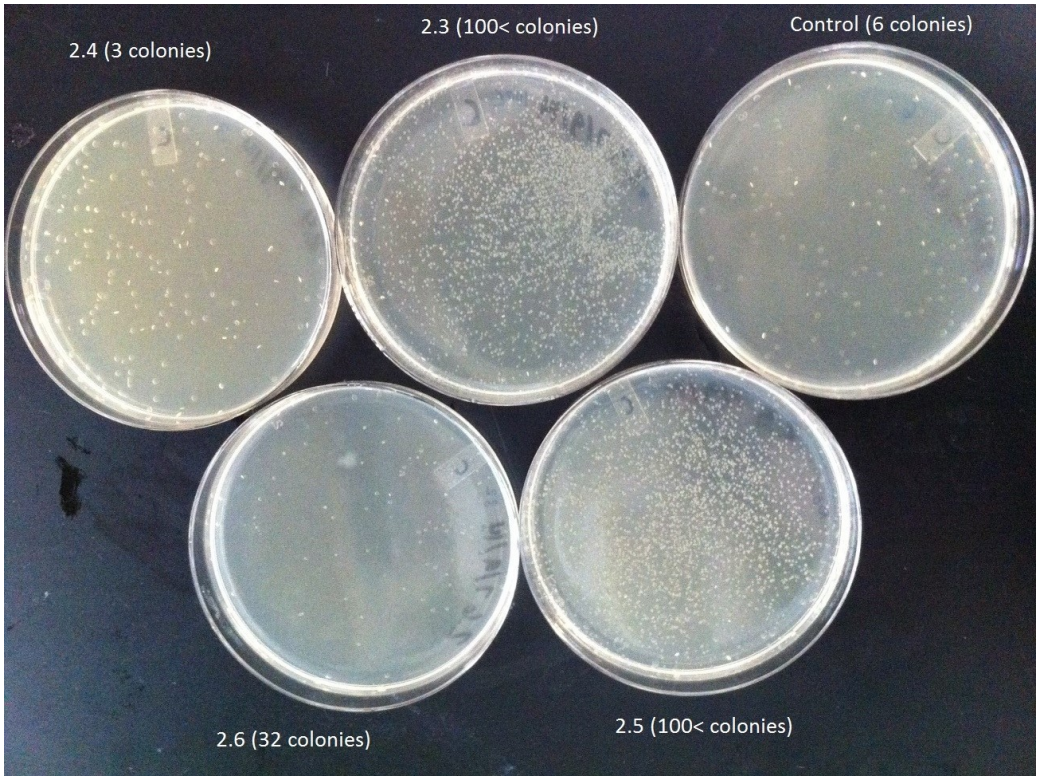

A
B
C
B2
B3
B4
B5
C3
C4
D2
D3


2
3
4
5
6
7
B3
B4
B5
B6
C2
C3
C4
C5
C6
C7
D4
D5
D6


6
7
8
9
10
B7
B8
B9
B10
B11
C7
C8
C9
C10
C11
D8
D9
D10

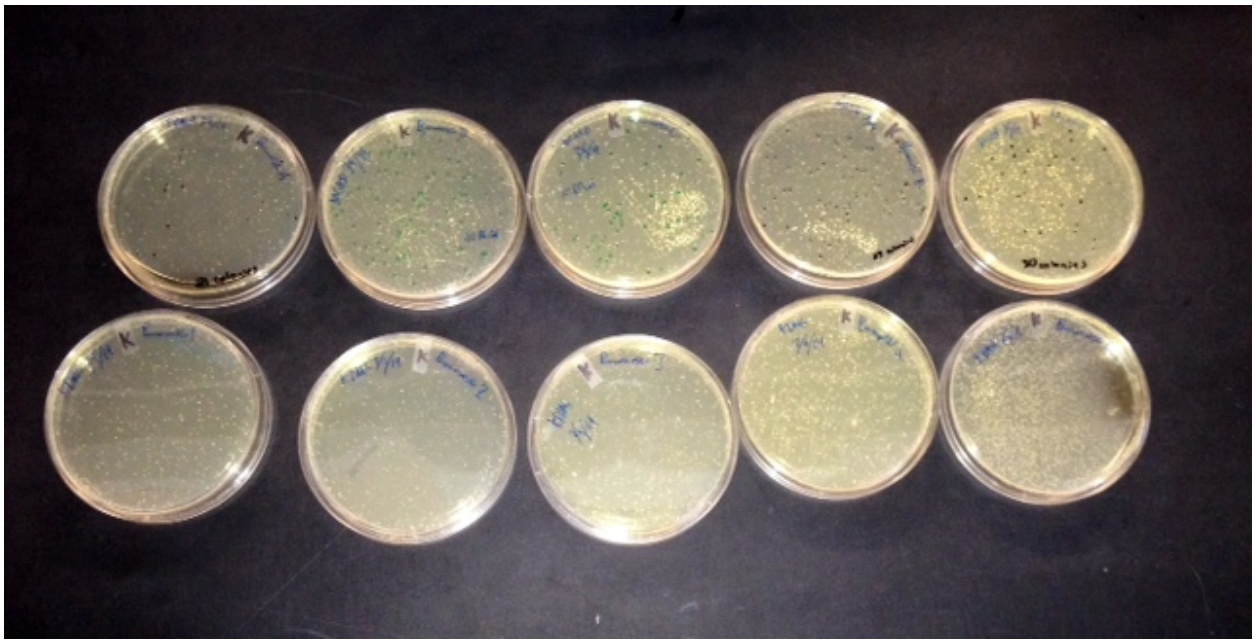
The plated transformations were also examined; they were successful, with the control plate of 2 yielding only 6 colonies and the control plate of 1 yielding 137, a low number relative to the other 1 plates.





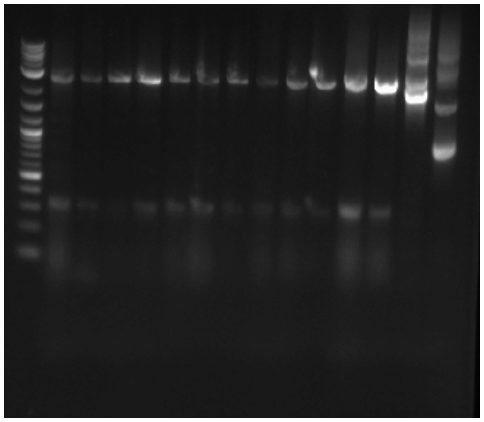
- Alex and Chloe also ran a colony PCR, choosing 5 different colonies from plate 2.3 and 5 different colonies from plate 1.6. However, this was not successful, as no bands were present when the gel was examined, not even in the control. Despite this, because the positive control PCR also turned out negative and the relatively low number of colonies on each control plate, 5 colonies were chosen from plate 2.3 and 10 colonies were chosen from plate 1.6 to grow in liquid culture. These will be examined the old way using minipreps and a test digest tomorrow.
- Kayla and Mike observed the ten transformation plates from the day before. There are noticeable colonies on each plate. A table of colony counts and a photo of all ten plates can be seen below. Because too many colonies grew on the control plate with no CIP, it was predicted that a majority of the colonies on the CIP- plates contained the plasmid ligated to itself rather than the plasmid containing the insert. On the other hand, the control plate for the CIP+ vector contained only 30 colonies. Thus, on a plate with 90 colonies, 2 out of every 3 colonies should contain a plasmid with the insert. To test this, colony PCR was done on 12 colonies from plate D, which contained 86 total colonies. For the 12 colonies, PCR tubes containing 15 uL of the PCR Megamix (112.5 uL Enzyme Master Mix, 1.5 uL 100 uM VF2, 1.5 uL 100 uM VR, and 184.5 uL dH2O) and a sample from the corresponding colony were prepared. The thermocycler was set to run at 98ºC for 2 minutes, followed by 35 times 98ºC for 30 seconds, 55ºC for 30 seconds, and 72ºC for 3 minutes, then 72ºC for the last 10 minutes. For more details, view the colony PCR protocol in the protocol folder.

  Plates 1-5 (Top) and A-E (bottom)



After the colony PCR was complete, all 14 samples were run on a gel, which can be seen below. According to the gel, all 12 colonies had an insert of about 2.5 kb. Therefore, any colony could be selected for liquid culture. Five liquid cultures were made later in the afternoon using colonies 1-5.

7/10/14 Colony PCR Gel



- Professor Farny and Mike met with Andy Butler to discuss a few aspects of the gas chromatography and mass spectroscopy device. We ran a few test samples, the results of which can be seen in the week 6 directory, labeled "**Initial GC/MS Trial**." A protocol outline is also available in the main protocol Directory, titled "**GC/MS Method Control Parameters.**"
- Corbyn and Shawna miniprepped the liquid cultures of the BCLA biobrick 3 and 5. We used the nanodrop to check the DNA concentration. Then, we ligated all 3 CAEV gel purified inserts into the chloramphenicol backbone, in the hopes of creating a CAEV biobrick. The table below shows the codes used to label each sample as well as the amount of insert we utilized in the ligation:

Code	Volume of Insert
1/2	2 µL CAEV1
1/5	5 µL CAEV1
1/10	10 µL CAEV1
2/2	2 µL CAEV2
2/5	5 µL CAEV2
2/10	10 µL CAEV2
3/2	2 µL CAEV3
3/5	5 µL CAEV3
3/10	10 µL CAEV3

We then sequenced all 5 promoter+RBS minipreps as well as the newly created BCLA biobrick 3 and 5 minipreps. When the CAEV ligation was complete, we transformed them and then plated them on cam plates, leaving them in the warm room overnight.

Also, as a team we had a meeting to prepare for the Women in Science day camp that is on Monday July 14th. The notes from this meeting can be found in the Women in Science folder on the google drive.

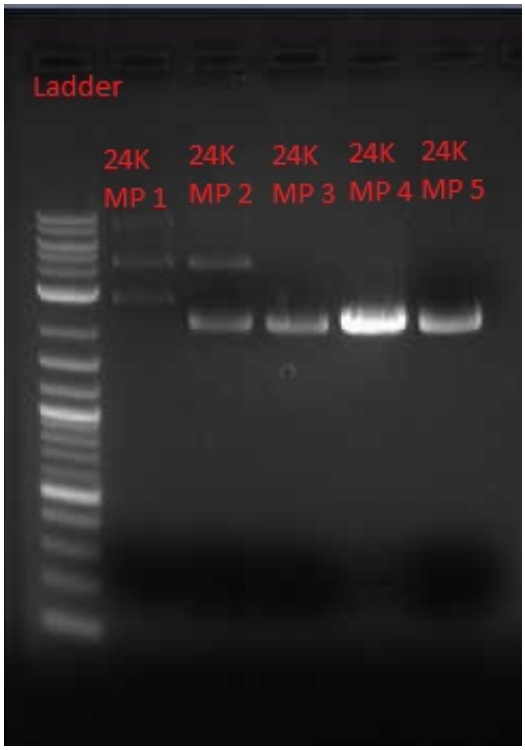
Day 29 - Friday- 07/11/14

- Kayla worked on the minipreps for 24K, the complete banana odor construct. The DNA concentrations of the five minipreps can be seen in the table below. After the minipreps were finished, all five samples were test digested with EcoRI and PstI. The 25 uL reactions were made up of 10 uL of miniprep DNA, 9.5 uL dH2O, 2.5 uL NEB Buffer 2, 1 uL BSA, 1uL EcoRI, and 1 uL PstI. Once the DNA had digested for two hours, 5uL of 6x loading dye were added to each reaction tube and the samples were loaded into a gel. The gel was run at 95V for 30 minutes to see if the complete insert was present. Based on the gel, none of the digested minipreps contained the full insert. In the lanes containing DNA from minipreps 1 and 2, there are multiple bands present, but none of them are the 2300 bp insert. As for minipreps 3-5, only one band of plasmid DNA can be seen on the gel. Therefore, ligation of the promoter to the RBS-ATF1-DT construct must be repeated. Gel purification (option 2) will be used to reach the desired product because 3A assembly has failed twice already.

  24K Miniprep Concentrations (ng/uL)

Miniprep 1	153
Miniprep 2	213.5
Miniprep 3	97.5
Miniprep 4	154.4
Miniprep 5	183

7/11/14 Test Digest



In addition to miniprepping and test digesting, an attempt was made to quantify the agglutination assay. Three tubes with various combinations of *E. coli*, 5% BSA in PBS, and *E. coli* antibody were prepared. All tubes contained 600 uL of *E. coli* culture and 600 uL of 5% BSA in PBS. No antibody was added to tube A, 6 uL of antibody were added to tube B, and 0.5 uL of antibody were added to tube C. The initial OD of each sample was measured and then the tubes were rotated for an hour to allow for the agglutination to occur. Following the agglutination, the liquid from each tube was divided among 3 30 micron spin columns. The columns were supposed to be spun for 1 minute at 1500 rpm, but they were accidentally spun at 15000 rpm. Because 15000 rpm is the max speed of the centrifuge, any clumps that formed during the agglutination process were most likely broken up and the samples had to be discarded.

- Professor Farny and Michael created differing liquid samples to use in testing GC/MS specifications, the can be seen in the table below:

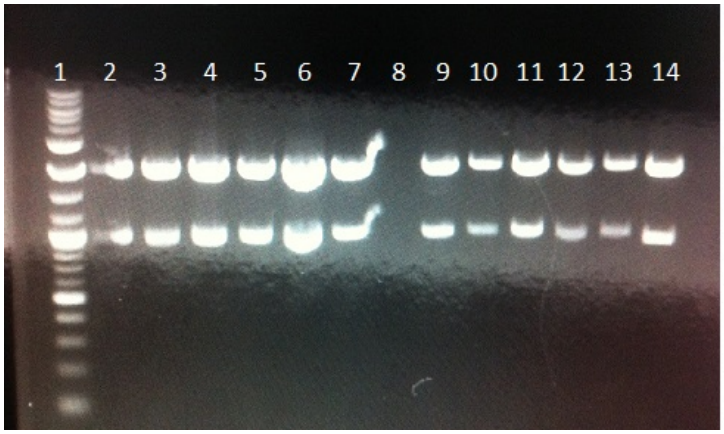
Gas Chromatography/ Mass Spectroscopy Variable Sample Run

Vial	Contains
1	LB (Naive)
2	LB + IAlc @5mM
3	LB + IAce @5mM

4	LB + IAlc + IAce both @5mM
5	LB from Cell Growth
6	LB + IAlc+IAce both @5mM from Cell Growth
7	LB + IAlc + IAce both @5mM -> 2x LB dilution
8	LB + IAlc + IAce both @5mM -> 10x LB dilution
9	LB + IAlc + IAce both @5mM -> 50x LB dilution
10	LB + IAlc + IAce both @5mM -> 100x LB dilution
11	LB + IAlc + IAce both @5mM ->1000x LB dilution
12	Rerun of LB sample frozen @ -80C on 07/09/14

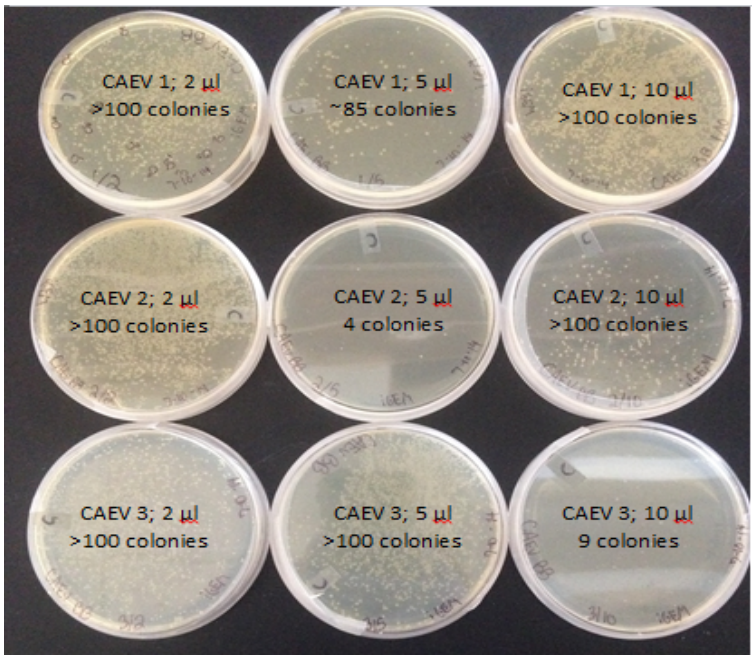
- The results of the "**Gas Chromatography/ Mass Spectroscopy Variable Sample Run**" are located in the Week 6 main directory.

- Chloe prepared a miniprep of the liquid cultures grown successfully overnight (2.3A, 2.3B, 2.3C, 2.3D, 2.3E, 1.6A, 1.6C, 1.6D, 1.6E, 1.6G, 1.6H), checked their concentrations on the nanodrop, prepared a test digest of all samples, and ran them on a gel. (The culture of 1.6G was very promising, as it was significantly more green than all other cultures, though every 1.6 culture was much more green than every 2.3 culture). The gel proved that all of the ligations were successful.

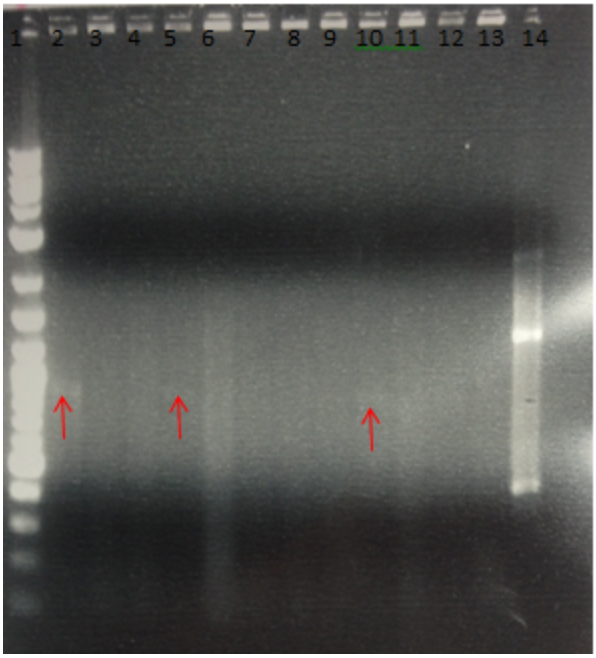


Lane	Contents
1	Ladder
2	18C+19C (1) From Plate 1.6
3	18C+19C (2) From Plate 1.6
4	18C+19C (3) From Plate 1.6
5	18C+19C (4) From Plate 1.6
6	18C+19C (5) From Plate 1.6
7	18C+19C (6) From Plate 1.6
8	Empty
9	19C+20C (1) From Plate 2.3
10	19C+20C (2) From Plate 2.3
11	19C+20C (3) From Plate 2.3
12	19C+20C (4) From Plate 2.3
13	19C+20C (5) From Plate 2.3
14	GFP Insert Control

- Shawna and Corbyn made modifications to the rainbow gel procedure to avoid using the TAE buffer. Instead, they created a 1% gel using water in both the gel and the chamber. Two trials resulted in the ideal protocol being running a 1% gel at about 150V for 8-10 minutes. They also analyzed their sequencing of the BCLA biobrick and promoter+RBS. Using the sequencing data they confirmed the BCLA biobrick and found the Promoter+RBS ligation to be a failure. In troubleshooting the promoter/RBS ligation error they discovered a possible mistake in miniprep labels, causing the double terminator to be ligated into the construct. Thus, they sent out 1C (DT) for sequencing to see if there was a mix up with the minipreps (possible switch between promoter and terminator). We also sent out the RBS (4A) for sequencing, but that was by mistake because we intended to send the promoter (5A). The growth on the CAEV plates from the previous day was very high, and a picture showing the labels and colony counts can be seen below.



12 colonies were selected from the 1/2 CAEV plate and analyzed via colony PCR and gel electrophoresis.The resulting gel can be seen below with labeled lanes. It appears as if lanes 2,5,and 10 contain the correct CAEV insert (although the band is very light we still think it is there). Lane 14 is a control, which was the Promoter-Ribosome sample 5. The purpose of the control (which had a known size) is to check that the PCR worked as well as to have an idea of how much the primers increases the size of the insert of interest.



Lane	Contents
1	Ladder
2	CAEV colony 1
3	CAEV colony 2
4	CAEV colony 3
5	CAEV colony 4
6	CAEV colony 5
7	CAEV colony 6
8	CAEV colony 7
9	CAEV colony 8
10	CAEV colony 9
11	CAEV colony 10
12	CAEV colony 11
13	CAEV colony 12
14	Control